THE ORIGIN OF CIGUATERA

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Ciguatera is caused by eating fish contaminated with ciguatoxins. Ciguatoxins-1, -2 and -3 are the major ciguatoxins found in the flesh and liver of ciguateric fishes with ciguatoxin-1 the most toxic and most abundant. Gambiertoxin-4b is the likely precursor of ciguatoxin-3 which is in turn oxidatively metabolised in fishes to ciguatoxin-1. Consequently, gambiertoxin-4b accounts for more than 90% of the toxicity of ciguateric fishes. Gambiertoxin-4b has been extracted from biodetritus containing large numbers of the benthic dinoflagellate *Gambierdiscus toxicus*, indicating that *G. toxicus* is the primary source of toxins involved in ciguatera. Putative gambiertoxins have also been detected in certain strains of cultured *G. toxicus*. However, the link between *G. toxicus* and ciguatera remains circumstantial since gambiertoxin-4b has not yet been unambiguously identified from cultures of this dinoflagellate.

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Ciguateric fishes are poisonous because their flesh and viscera contain elevated concentrations of lipid-soluble polyether ciguatoxins (Murata et al.,1990; Lewis et al.,1991; Lewis & Sellin, 1992). Ciguatoxins-1, -2 and -3 (Fig.1) have been isolated fromthe flesh of toxic carnivorous fish with ciguatoxin-1 being most abundant and most toxic (Lewis & Sellin,1992). Some minor toxins (presumably ciguatoxins) remain to be characterised from carnivorous and herbivorous fishes (Murata et al.,1990; Lewis et al.,1991; Lewis & Sellin,1992; Legrand et al., 1992). Ciguatoxins-2 and -3 do not have the secondary hydroxyl on carbon 54 and are therefore less-polar than ciguatoxin-1 (Lewis et al., 1991). Ciguatoxin-3 is thought to be an intermediate in the oxidative metabolism of a less-polar precursor, gambiertoxin-4b, to cigua- toxin-1 (Lewis et al., 1991) whereas ciguatoxin-2 is a diastereomer of ciguatoxin-3 (Lewis et al., 1993) which may originate from a different precursor. The stereochemistry at carbon 52 indicates that ciguatoxin-2 has a different structural backbone to ciguatoxins-1 and -3 (Lewis et al., 1993). Ciguatoxins-2 and -3 induce similar bioassay signs in mice, including hind limb paralysis, the only bioassay sign that differentiates these less-polar ciguatoxins from ciguatoxin-1 (Lewis et al.,1991). Precursors of ciguatoxins-2 and -3 are thought to enter the marine food web incidentally upon ingestion by lower trophic level fishes (e.g. herbivores/ detritivores like surgeonfishes (Randall,1958; Lewis et al.,1991)) or invertebrates (Kelly et al.,1992; Lewis et al.,1994). These species are in turn preyed on by carnivorous fishes.

Randall's (1958) hypothesis that the ciguatera toxins originate from a small benthic organism received strong but circumstantial support when Yasumoto et al. (1977a,b) extracted ciguatoxin-like and maitotoxin-like toxins from a benthic detrital sample containing large numbers of the dinoflagellate *Gambierdiscus toxicus* Adachi & Fukuyo. Yasumoto et al. (1979a) and Bagnis et al. (1980) were able to repeat the extraction of such toxins from biodetritus from the Gambier Islands and to extract maitotoxin from cultures of *G. toxicus*. However, the column and thin-layer chromatography of the ciguatoxin-like toxin found by Yasumoto's group was not consistent with the major ciguatoxin (ciguatoxin-1) found in fishes but instead was indicative of a less-polar ciguatoxin-like toxin (Lewis,1985). The role of *G. toxicus* in ciguatera remained in doubt since meagre amounts of this ciguatoxin-like toxin, if any, were produced by cultured *G. toxicus* (Yasumoto et al.,1979a; Bagnis et al.,1985a; Holmes et al.,1990; Murata et al.,1990; Yasumoto, 1990) and this toxin could not be extracted from wild *G. toxicus* outside of French Polynesia (Gillespie et al.,1985a). It was not until gambiertoxin-4b (precursor of ciguatoxins-1 and -3) was extracted from biodetritus samples containing large numbers of wild *G. toxicus* (collected in 1979 from the Gambier Islands and kept at -20° for many years) and its structure compared with that of ciguatoxin-1, that *G. toxicus* was once again considered the likely origin of ciguatera (Murata et al.,1990; Legrand et al.,1990,1992). Nine gambiertoxins have since been extracted from wild *G. toxicus*, including gambiertoxin-4c

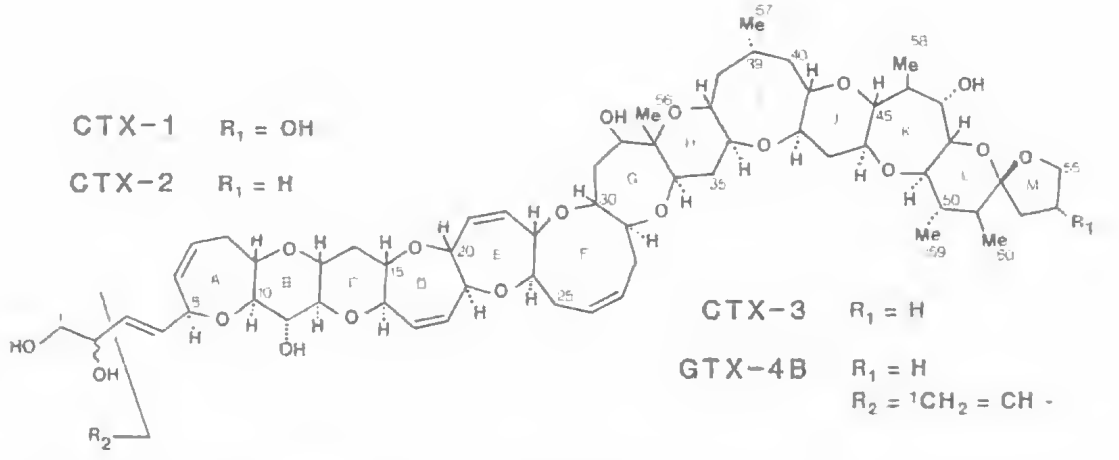

FIG. 1. Structures of ciguatoxins (CTX)-1, -2 and -3 and gambiertoxin-4b (GT-4b) (Murata et al.,1990; Lewis et al.,1991). Ciguatoxin-2 is a diastereomer of ciguatoxin-3 (Lewis et al.,1993).

(the major toxin in terms of mouse lethality) and the isomers, gambiertoxin-4a and -4b (Legrand et al.,1992). Of these, only the structure of gambiertoxin-4b is known (Murata et al.,1990). However, gambiertoxin-4b appears to be the most important gambiertoxin contributing to ciguatera as its oxidative products, ciguatoxin-1 and -3, account for more than 90% of the toxicity of ciguateric fish (Lewis et al.,1991; Lewis & Sellin,1992). The site of biotransformation of gambiertoxin-4b to ciguatoxin-3 and of ciguatoxin-3 to ciguatoxin-1 remains to be established, but likely occurs in the liver of fishes (Lewis et al.,1991).

Extraction of gambiertoxins from reef biodetritus containing wild *G. toxicus* is circumstantial evidence that *G. toxicus* is the origin of the toxins that cause ciguatera. Further support for this hypothesis was obtained by Holmes et al. (1991) and Holmes & Lewis (1992) who found two putative gambiertoxins (called major and minor based upon their relative contribution to total lethality) in cultures of certain strains of *G. toxicus*. These toxins were less-polar than ciguatoxins-1, -2 or -3 but were considered closely related to the ciguatoxins since: (i) both gambiertoxins produced bioassay signs in mice similar to those produced by ciguatoxins-2 and -3, (ii) the major (more-polar) toxin was shown to be a Na^+ channel activator toxin (as are the three ciguatoxins) that produced pharmacological responses in isolated tissues similar to those produced by the ciguatoxins (especially ciguatoxin-3; Lewis & Wong Hoy,1993) and (iii) the major toxin competitively inhibited the binding of [^{3}H]brevetoxin-3 to rat brain synaptosomes. The ciguatoxins (and brevetoxins) are the only toxins known to competitively inhibit brevetoxin binding to the Na^+ channel (Lombet et al.,1987; Lewis et al.,1991). However, gambiertoxin-4b has not been unambiguously identified from cultured *G. toxicus* and therefore the origin of the precursor of ciguatoxins-1 and -3 remains to be established. Many reports have claimed to extract ciguatoxin or ciguatoxin-like toxins from cultures of *G. toxicus* (Bergmann & Alam,1981; Withers,1982; Shimizu et al.,1982; Miller et al.,1984; Lechat et al.,1985; Durand et al., 1985; Durand-Clement,1987) but these investigations relied upon a liquid-liquid partition (eg. diethyl ether-water) to completely separate ciguatoxin-like toxins from the considerable amounts of maitotoxin present in crude extracts. Since maitotoxin can partition into both the lipid- and water-soluble phases (Yasumoto et al., 1979a; Holmes et al.,1990) these former studies are unlikely to have completely separated any ciguatoxin-like material from the maitotoxin.

Production of gambiertoxins in cultured *G. toxicus* appears to be strain-dependent, with most clones only producing maitotoxins (Holmes et al.,1991). These authors found that only two of 13 cultured strains produced putative precursors of the ciguatoxins, indicating that the actiology of ciguatera is likely restricted to genetic strains of *G. toxicus* which can produce gambiertoxins. The most striking evidence for this genetic variability was that putative gambiertoxins were produced by only one of four *G. toxicus* clones

isolated from the same site, with only one of two clones isolated from the same site and at the same time producing gambiertoxins (Holmes et al.,1991). This result has important implications for ecological studies of *G. toxicus*, as it indicates that the size of *G. toxicus* populations does not necessarily reflect the potential for these populations to cause ciguatera. Wild populations of *G. toxicus* from the Gambier Islands, Kiribati and Platypus Bay, Queensland have been found to produce gambiertoxins (Legrand et al.,1990, 1992; Holmes et al.,1991; Holmes et al.,1994) whereas a large population of wild cells from Flinders Reef, south Queensland did not produce detectable levels of these toxins (Gillespie et al., 1985a; Lewis et al.,1988a).

Only relatively low concentrations of gambiertoxins have so far been detected from cultured compared with wild *G. toxicus* cells (Holmes et al.,1991,1994; Holmes and Lewis,1992). Considerable variation can also occur in the concentration of gambiertoxins produced by wild *G. toxicus* (Holmes et al.,1994). Holmes et al. (1994) have proposed the existence of "super-producing strains" of *G. toxicus* to explain some of this variation in gambiertoxin production between cultured and wild *G. toxicus*. However, environmental factors are also likely to affect toxin production since the concentration (or type) of gambiertoxin produced can change in culture (Holmes & Lewis,1992). Environmental conditions obviously effect the growth of *G. toxicus* as they do for any other algae, but it is not known if environmental parameters can selectively affect the growth and toxicity of super-producing strains over non-producers. It is quite likely that the conditions which enhance growth will not necessarily be the conditions which enhance toxin production. Future research could focus on the effect of different combinations of genetic and environmental parameters on the rate of gambiertoxin production.

Ecological studies need to also consider the effect of different rates of turnover of *G. toxicus* populations. A large standing crop of *G. toxicus* does not necessarily indicate a greater potential to cause ciguatera compared with a smaller population, if the lower biomass is simply a reflection of higher productivity *and* higher rates of consumption by herbivores. Recent evidence that fishes can excrete/metabolise the ciguatoxins (Tosteson et al.,1988; Lewis et al.,1992) suggests that considerably greater quantities of gambiertoxins are entering the marine food web than would otherwise be expected by the frequency of ciguatera. Seasonal patterns of ciguatera in some Pacific Island countries (Sorokin, 1975; Dawson,1977; Bagnis,1979; Bagnis et al.,1992; Lewis,1992) may also reflect seasonal patterns in the abundance and/or gambiertoxin production by wild *G. toxicus*. There are numerous reports of seasonal variation of populations of *G. toxicus* and other benthic dinoflagellates (Yasumoto et al.,1979b; Carlson & Tindall,1985; Gillespie et al.,1985b; Bagnis et al., 1985b; Ballantine et al., 1985,1988; Bomber et al., 1988; McCaffrey et al., 1992; Holmes et al.,1994). However, seasonal patterns of *G. toxicus* abundance have been correlated with fish toxicity only in French Polynesia (Bagnis et al.,1985b).

Most strains of *G. toxicus* appear not to produce gambiertoxins, while most produce a maitotoxin (Holmes et al.,1991). Maitotoxins are generally referred to as water-soluble toxins although they are soluble in a range of organic solvents and a butanol-water liquid-liquid partition will recover nearly 100% of maitotoxin in the butanol phase (unpublished result). The maitotoxins have a cyclic polyether structure as do the gambiertoxins and ciguatoxins (Yokoyama et al.,1988; Murata et al.,1991, 1992, 1993). Interestingly, the type of maitotoxin produced by *G. toxicus* is dependent upon the strain being cultured, with each strain apparently producing only the one type of maitotoxin (Holmes et al.,1990). Holmes & Lewis (in press) have recently found that large maitotoxins (maito-toxins-1 and -2, with molecular weights >3,000) were produced by strains of *G. toxicus* which do not produce gambiertoxins, whereas the small maitotoxin-3 (molecular weight 1,060 for the disodium salt) was produced by a clone which also produces gambiertoxins. The molecular weight of maitotoxin-3 is the same as gambiertoxins-4a and -4b (Murata et al.,1990; Legrand et al.,1992). Holmes & Lewis (in press) have suggested that the biosynthesis of gambiertoxins and maitotoxins may be linked in strains of *G. toxicus* which produce both of these types of toxins.

Maitotoxin has been found in the gut contents of surgeonfishes (Yasumoto et al.,1976) but there is little evidence that maitotoxin is accumulated in the flesh of these or other fishes. Water-soluble, maitotoxin-like toxins have been extracted from the flesh of fishes in Hawaii and Queensland (Iwaoka et al.,1993; Endean et al., 1993). However, these studies based the detection of these toxins, at least in part, on intraperitoneal (i.p.) injections into mice of at least 100 mg of crude extracts (≥5 g extract/kg mouse

body weight). Doses of crude fish extracts >1 g/kg i.p. can produce non-specific toxic effects (Banner et al.,1961; Lewis et al.,1988a). Unsaturated fatty acids extracted from shellfish have also been shown to produce toxic effects when injected i.p. into mice (Takagi et al.,1984). Calculations of total toxicity based upon lethal doses of such large amounts of extract would likely result in the overestimation of the quantity of toxin present. Additionally, the water-soluble extracts of fish flesh killed mice quickly (5 and 13 min, Iwaoka et al.,1993; 3–30min, Endean et al., 1993). However, based upon these rapid deaths, we conclude that any water-soluble toxins isolated were not maitotoxins, since very large doses of native maitotoxin (e.g. >100 lethal units) would be required to produce such short death times. The three maitotoxins characterised so far are potent but slow acting toxins with the shortest survival times (calculated according to Molinengo, (1979)) being greater than 41 min (Holmes et al.,1990; Holmes & Lewis in press).

Maitotoxins are the most toxic toxins produced by *G. toxicus*, often comprising more than 99% of total toxicity (Holmes & Lewis,1994). Fishes fed cultured *G. toxicus* cells display abnormal swimming behaviour (Davin et al.,1986,1988; Kelly et al.,1992) probably as the result of maitotoxin intoxication. Maitotoxin poisoning of fishes in the wild may result in these fishes being preferentially preyed upon. This could be a mechanism for concentrating gambiertoxins through the food chain of fishes when herbivorous fishes ingest strains of *G. toxicus* which produce both gambiertoxins and maitotoxins.

Toxins other than ciguatoxins-1, -2 and -3 have been suggested as causes of ciguatera. Scaritoxin, extracted from parrotfish (*Scarus gibbus*) from the Gambier Islands (Chungue et al.,1977), may be a less-polar form of ciguatoxin (Lewis et al., 1991; Legrand et al., 1992). Vernoux & Talha (1989) detected fast-acting ciguatoxins in fish flesh; instability and quick death-times induced by these toxins distinguish them from ciguatoxins-1, -2 and -3, which are stable and slow acting (Lewis et al.,1991). Other toxins suggested as causal agents of ciguatera include palytoxin, okadaic acid and other toxins (predominately water-soluble toxins) produced by the benthic dinoflagellate species *Ostreopsis* spp. and *Prorocentrum* spp., and toxins produced by the planktonic cyanophyte (cyanobacterium) *Oscillatoria* (*Tricodesmium*) *erythraea* (Yasumoto et al.,1980; Nakajima et al.,1981; Murakami et al., 1982; Tindall et al.,1984,1990; Norris et al.,1985; Holmes et al.,1988; Kodama et al., 1989; Dickey et al.,1990; Juranovic & Park,1991; Hahn & Capra,1992).

Palytoxin is a potent water-soluble toxin isolated from various *Palythoa* coral species (Moore & Scheuer,1971; Habermann,1989). Palytoxin (or one of its congeners) has been found in the flesh and viscera of triggerfish *Melichthys vidua* (Fukui et al.,1987a,b) and smoked mackerel *Decapterus macrosoma* (Kodama et al.,1989). Palytoxin is also thought to be responsible for intoxications caused by eating parrotfish liver (*Ypsiscarus ovifrons*) from western Japan (Noguchi et al.,1987). The extent of human poisoning from palytoxin is not known but we believe it is a separate poisoning distinct from ciguatera. Hospitalised cases present with signs distinguishable from ciguatera including elevated serum enzyme levels (Noguchi et al.,1987; Kodama et al., 1989). However, mild palytoxin poisoning may be mistaken for ciguatera.

Okadaic acid is a lipid-soluble polyether toxin with similar chromatography to ciguatoxins (Yasumoto et al.,1980; Murakami et al.,1982). Okadaic acid was originally isolated from the black sponge *Halichondria okadai* (Tachibana et al.,1981) but has been isolated from the benthic dinoflagellates *Prorocentrum lima* (Murakami et al.,1982) and *P. concavum* (Dickey et al.,1990) and from the temperate dinoflagellates *Dinophysis* (Lee et al.,1989). Okadaic acid is one of the toxins that can accumulate in shellfish to cause a disease known as diarrhetic shellfish poisoning (Lee et al.,1988). However, the only fishes from which okadaic acid has been extracted are barracuda from the Caribbean (Gamboa et al.,1992). This result requires confirmation, including an estimate of whether the levels detected were sufficient to cause human poisoning. The primary basis for linking okadaic acid (and the other toxins produced by *Ostreopsis* spp. and *Prorocentrum* spp.) with ciguatera is (i) the dinoflagellates that produce these toxins would likely be ingested by the same or similar herbivores that ingest *G. toxicus*, and (ii) the diverse range of symptoms of ciguatera could result from a combination of several toxins. However, there is little evidence to indicate that the toxins produced by these dinoflagellates accumulate in fish flesh to cause human poisoning. Symptoms of ciguatera could result from ingestion of different relative amounts of different ciguatoxins (Lewis & Sellin,1992) and/or different doses of

ciguatoxin (Yasumoto et al.,1984; Lewis et al., 1988b).

Evidence linking *O. erythraea* to ciguatera is similarly unconvincing. *O. erythraea* is a tropical and sub-tropical planktonic, filamentous cyanophyte common off the east and west coasts of Australia (Hallegraeff,1991). However, this species is also common in areas where ciguatera has not been reported (Lewis,1988; unpublished observations). Toxins have been extracted from Caribbean and Australian samples of *O. erythraea* (Hawser et al.,1991; Hahn & Capra, 1992) but plankton-feeding fishes, which would be the most likely to ingest this algae, apparently do not cause ciguatera (Randall,1958). Hahn & Capra (1992) showed that a toxic fraction could be briefly accumulated by filter-feeding bivalves and that this toxin may be subsequently accumulated in the viscera of the molluscivorous fish *Trachinotus blochi*. However, only one case of human poisoning by *Trachinotus* sp. has been recorded in Australia since 1965 (unpubl. data).

Ciguatera is caused by eating fish which have accumulated toxins from their diet. Nearly all benthic dinoflagellates produce toxins but not all of these toxins are accumulated to harmful levels in the flesh of fishes. Evidence to-date suggests that, of the benthic dinoflagellates, only *G. toxicus* produces toxins, especially gambiertoxin-4b, responsible for the human poisoning syndrome known as ciguatera. The ciguatoxins are the major toxins found in ciguateric fishes and must be considered the primary cause of ciguatera. Other toxins and other sources of these toxins have been suggested as being involved in ciguatera but their involvement, if any, remains to be established.

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